MOLECULAR DETECTION OF SOME VIRULENCE CHARACTERS OF PROTEUS SPECIES

Amira R. El-Baz[1], Heba E. El-Degla[2*], Gamal M. Abdel-Fattah[1]

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Abstract

Numerous virulence factors, which are in turn controlled by virulence genes that are produced in operons, contribute to the virulence of proteus isolates. This study aimed to detect genes mrp, flaA, ureC, and zapA in 25 proteus isolates by using the polymerase chain reaction (PCR). In this work, isolated proteus species were identified via molecular analysis. Twenty-five isolates samples from patients getting care at Mansoura University Hospitals were collected between October 2019 and February 2021. Biochemical testing on all of the samples found positive isolates of proteus species were done. The full sequences of the genes examined in this study were amplified using a PCR to detect the virulence traits of proteus species. The precise primers (QIA amp kits) were used for zapA, ureC, mrp, and flaA amplification. After the study, PCR amplification was used to identify the genes responsible for proteus isolates virulence and according to agarose gel electrophoresis. The results of the gene's amplification products were shown that 25 proteus isolates had (100%) positive results of virulence genes zapA, ureC, mrp, and flaA by using a PCR detection technique. The virulence of proteus isolates was regulated by genes, and PCR amplification revealed that proteus isolates had the genes mrp, flaA, ureC, and zapA, which cause crack toxicity, respiratory tract infections, and together public-acquired and drain-associated urinary tract infections (UTIs).

Keywords: proteus isolates virulence, virulence genes, proteus infections, PCR.

[1] Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt

[2] Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

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INTRODUCTION

In the family Enterobacteriaceae, the genus proteus includes rod-shaped Gram-negative bacteria (Brenner *et al.,* 2005). They are widely dispersed throughout the ecosystem, mainly in the water, soil, and gastrointestinal tracts of people and animals (Drzewiecka, 2016). A nosocomial, opportunistic pathogen called proteus is also prevalent in infections picked up in the community (Pearson *et al.,* 2010).

Proteus mirabilis and *Proteus vulgaris* have each been connected to crack toxicity, respiratory tract infections, and collectively public-acquired and drain-associated urinary tract infections (UTIs) (Li, *et al.,* 2002). *P. mirabilis* possesses a wide range of morphological and pathogenic characteristics, such as the ability to manufacture enzymes and toxins as well as the ability to fly on particular dense culture media and attach to human epithelial cells utilizing non-agglutinating fimbriae. The virulence of Proteus spp. is influenced by a variety of virulence factors, which are in turn regulated by virulence genes produced in operons (Manos *et al.,* 2004).

It has been established that the proteus infection's virulence agent, urease, is responsible for the formation of kidney and bladder stones in urolithiasis. Urease is produced by the ureA, ureB, ureC, ureD, ureE, ureF, and ureR genes on the ure operon; earlier research has identified ureC as a crucial gene that codes for the enzyme's largest subunit and also contains the enzyme's catalytic region (Li & Mobley, 2002). The urea substrate enzyme is finally broken down by urease. The four genes that make up the zap operon, zapA, zapB, zapC, and zapD, are essential for the production of IgA protease, which is one of the protease products. ZapA is also a key component of the zap operon.

There are several different types of fimbriae and haemagglutinins in *P. mirabilis*, but manose resistant/proteus like fimbriae (MR/P) is the most well-known. The mrpA gene is essential to pathogenicity since it provides numerous virulence features such as bacterial adherence to epithelial tissue, biofilm formation, and swarming phenomena (Rózalski *et al.,* 1997). Its presence has been

connected to the development of pyelonephritis.As part of the steps that transform a swimmer cell into a swarmer cell, the rate of particular proteins being synthesized noticeably rises.

The protein most visible in charge of swarming is flagellin. The flaA gene produces the primary flagellin, FlaA (Belas, 1994). To get through the immune system during a UTI or to give motility in difficult situations, functional recombinant flagella may be employed. The ureC, zapA, flaA, and mrpA genes were the focus of this investigation. Taking into account that they have been demonstrated to be more common in earlier studies and are known to code the most important virulence factors (Alsherees *et al.,* 2016). This study used a polymerase chain reaction to evaluate the molecular aspects of virulence in proteus species (PCR).

METHODOLOGY

Clinical samples 25 proteus isolates, such as wounds, urine, stool, nose swab, and sputum, were collected from patients admitted to various Mansoura University Hospitals. In sterile settings, samples were taken, and they were then cultivated on blood, MacKonkey׳s agar media, and cystine lactose electrolyte deficient (CLED) agar media. Gram stains were performed on all sample after 24 hours of incubation at 37 ^{0}C. For additional verification of the samples of proteus species, biochemical identification was performed on all the samples with the same method.

Detection of proteus virulence genes mrp, flaA, ureC, and zapA by using PCR

Isolation of genomic DNA from proteus species

Genomic DNA was extracted using the DNA extraction kit (QIAGEN) by the manufacturer׳s instructions. Centrifuging was done on the microorganisms in pellet form at 5000 x g for 10 min (7500 rpm). In 180 µl of the appropriate enzyme solution (20 mg/ml lysozyme or 200 mg/ml lysostaphin; 20 mM Tris. hydrochloric acid (HCL), pH 8.0; 2 mM ethylenediaminetetraacetic acid (EDTA); 1.2% Triton), was added to the bacterial pellet. The bacterial pellet was incubated for at least 30 minutes at 37 ^{0}C. 20 ml of proteinase K and 200 ml of Buffer AL were added and combined for 30 minutes at 56 ^{0}C, followed by 15 further minutes at 95 ^{0}C, which was the recommended incubation time.

Identification of mrp, flaA, ureC, and zapA genes by using PCR

Several virulence genes such as (mrp, flaA, ureC, and zapA) were discovered using conventional PCR amplification. **Table** No. (1) presents the primers used to identify the mrp, flaA, ureC, and zapA genes, whereas **Table** No. (2) shows the amplification process (Al-Mayahi, 2017).

Table1. The primer sets were used for PCR assay in this study.

Type	Gene name		Oligo sequence (5′-3′)	Product size (bp)	Reference
Virulence factors	ZapA	F	ACCGCAGGAAAACATATAGCCC	540	(Stankowska , et al., 2008)
		R	GCGACTATCTTCCGCATAATCA		
	UreC	F	CCGGAACAGAAGTTGTCGCTGGA	533	(Takeuchi et al., 1996)
		R	GGGCTCTCCTACCGACTTGATC		
	Mrp	F	ACACCTGCCCATATGGAAGATACTGGTACA	550	(Zunino et al., 2001)
		R	AAGTGATGAAGCTTAGTGATGGTGATGGTG ATGAGAGTAAGTCACC		
	flapA	F	AGGATAAATGGCCACATTG	417	(Ali & Yousif, 2015)
		R	CGGCATTGTTTAATCGCTTTT		

Table 2. PCR thermocycling programs and conditions.

Gene name	Temperature (C°)/Time					Cycle number
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
ZapA	**95/1 min**	**94/30 sec**	**53/1 min**	**72/1 min**	**72/5 min**	**35**
UreC	**94/3 min**	**94/1 min**	**63/30 sec**	**72/1 min**	**72/7 min**	**30**
Mrp	**94/3 min**	**94/1 min**	**40/1 min**	**72/1 min**	**72/5 min**	**30**
flaA	**95/3 min**	**95/30 sec**	**54.2/30sec**	**72/ 30 sec**	**72/5 min**	**30**

Detection of PCR products

Following amplification, PCR products totaling 10µl were separated on a 1.5% agarose gel using Tris/Borate/EDTA (TBE) buffer (pH 8). The gel was then stained with ethidium bromide and put through electrophoresis at 100 volts. A 7 mm lane of an agarose gel that was being utilized as a DNA marker received ten µl of "Simply Load TM 100 bp DNA ladder" (Lonza, USA). The gels were captured under ultraviolet light after electrophoresis on a gel.

RESULTS

This investigation used 25 samples from various clinical sources, including nasal swabs, wounds, urine, stool, and sputum. The findings were reported in **Table** No. (3). The gene detection findings were presented in **Table** No. (4) and revealed that 25 isolates of proteus species had (100%) positive results.

Table 3. Indicate the number and the type of proteus isolates.

Sample number	Type of sample
14	Wound
6	Urine
3	Stool
1	Nose swab
1	Sputum

Table 4. Indicate the zapA, ureC, mrp, and flapA genes in the most resistant proteus species isolates.

Gene detection	Proteus species isolates (n=25)	
	No	**%**
ZapA	25	100
UreC	25	100
MrP	25	100
FlapA	25	100

According to agarose gel electrophoresis results of the gene's amplification products, which showed up as amplicons with a molecular weight of 540 bp (Fig. 1), 25 isolates of proteus species were discovered to have (100%) of the zapA gene.

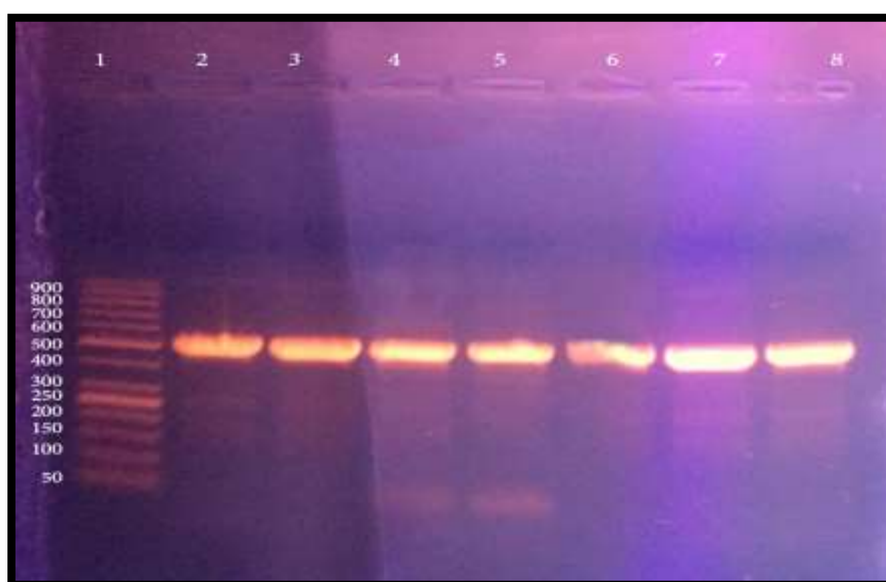

Fig. 1: zapA gene amplification products (540 bp) on agarose gel electrophoresis in isolates of proteus species. Lane 1: DNA Ladder (50 bp), Lane 2-8: positive results of zapA gene amplification products (540 bp).

When the ureC gene amplification products from the proteus species were processed on an agarose gel electrophoresis, the results revealed that 25 isolates (100%) carried this gene because they formed amplicons with a molecular weight of 533 bp (Fig. 2).

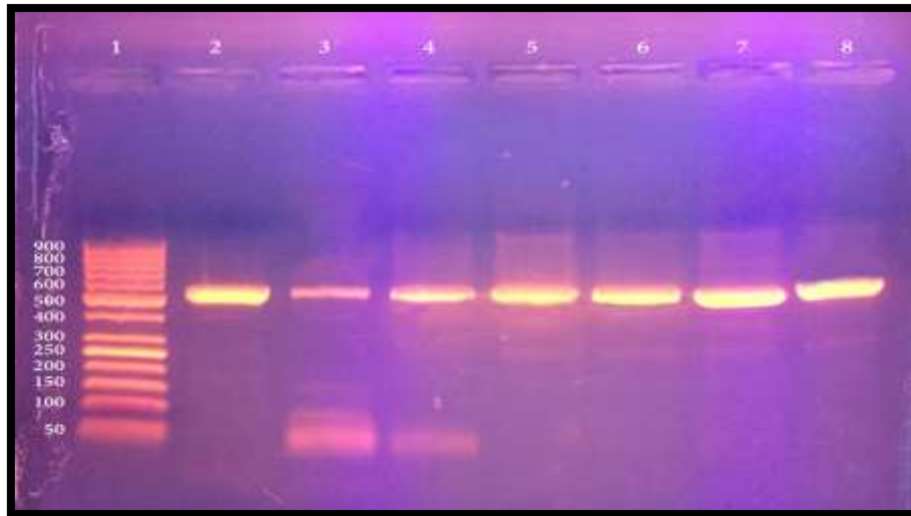

Fig. 2: ureC gene amplification products (533 bp) on agarose gel electrophoresis in isolates of proteus species. Lane 1: DNA Ladder (50 bp), Lane 2-8: positive results of UreC gene amplification products (533 bp).

The appearance of amplicons with a molecular weight of 550 bp observed on agarose gel electrophoresis of mrp gene amplification products of proteus species revealed that 25 isolates (100%) had this gene (Fig. 3).

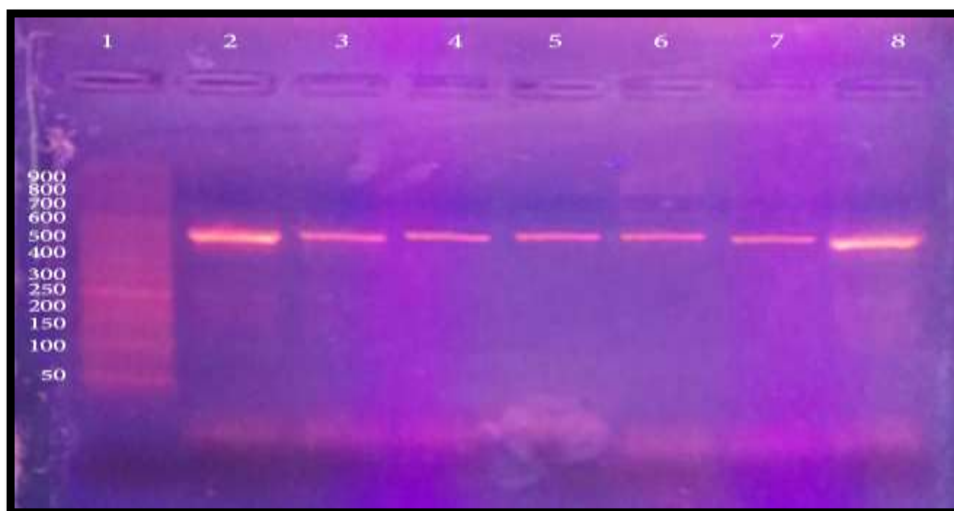

Fig. 3: mrp gene amplification products (550 bp) in the proteus species on agarose gel electrophoresis Lane 1: DNA Ladder (50 bp), Lane 2-8: positive results of mrp gene amplification products (550 bp).

According to the outcomes of agarose gel electrophoresis of the genes amplification products, which showed as amplicons with a molecular weight of 417 bp in (Fig. 4), 25 isolates (100%) of proteus species carried the flapA gene.

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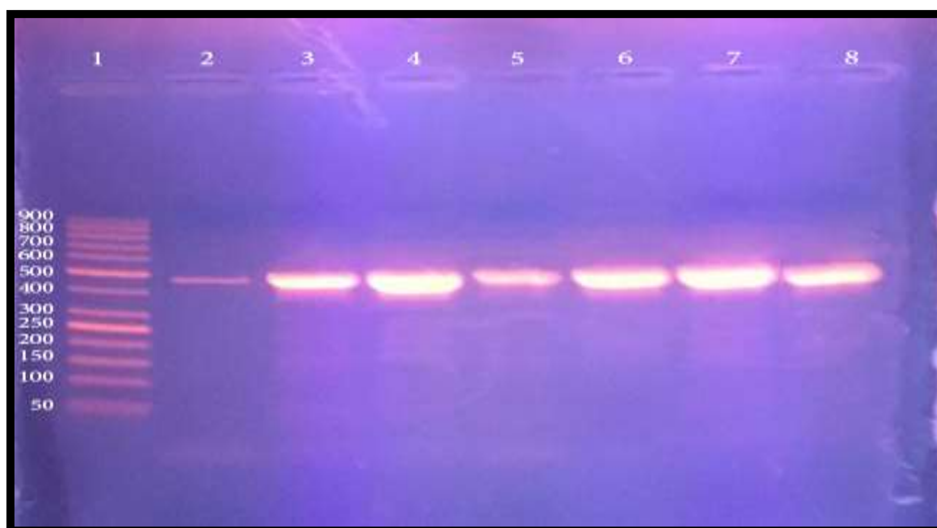

Fig. 4: flapA gene amplification products (417 bp) in the proteus species were electrophoresed on an agarose gel. Lane 1: DNA Ladder (50 bp), Lane 2-8: positive results of flapA gene amplification products (417 bp).

DISCUSSION

Based on the efficiency and speed of phenotypic findings measuring several virulence parameters, 25 isolates of proteus species were selected for genetic detection. Every clinically relevant source was present in these isolates. The manufacturer-recommended QIA amp Bacterial Genomic DNA Kit was used to extract the genome. To gauge the DNA concentration, a photometer was employed. The whole sequences of the genes examined in this study were amplified using a PCR. These genes were amplified using the particular primer pairs zapA, ureC, mrp, and flapA.

The findings of this investigation also revealed that the zapA gene, which was 100% responsible for protein synthesis, was present in all 25 proteus isolates. PCR was used to verify this, and the results were consistent with what (Bunyan & Albakery, 2020) discovered regarding *P. mirabilis*, which was that it had the zapA gene at a 1:1 (100 percent) ratio. Because it may destroy IgA and IgG antibodies, which lowers the immune response and makes the bacteria that produce them more hazardous, protease was regarded as one of the most crucial enzymes.

The ureC gene, which produces the urease enzyme, is thought to be a distinguishing characteristic of bacteria belonging to the proteus species. The results of the current study show that 25 proteus isolates at a rate of 100% contain the ureC gene. The findings were comparable with what (Ali & Yousif, 2015) found about *P. mirabilis*, which was that 29 isolates out of 30 isolates at a rate of 96.66% include the ureC gene.

The urease enzyme produced by *P. mirabilis* differs from the urease produced by other species of bacteria in that it is more active. It works by bringing the pH of the urine to a basic level, which results in the biofilm being formed by calcium and magnesium phosphate deposition. This leads to the development of Crystallin biofilm, a more complicated sort of biofilm that works to seal the catheter lumen and shield the bacteria from antibiotics, leading to treatment failure (Stickler, 2008).

The MR/P type, which encodes multiple chromosomal genes carried in two copies, is one of the most important fimbrial types. The first version is an operon made up of nine mrpABCDEFGHJ (mrp operon) genes necessary for the synthesis and assembly of the fimbria, while the second version is a single gene called mrpI that regulates the reproduction of operons. Urolithiasis starts as a build-up of bacteria from the proteus species in the bladder lumen, which promotes calcium ion deposition. This needs the presence of the enzyme urease and fimbria MR/P (Mirzaei *et al.,* 2019). The present study's findings demonstrate that the mrp gene is present in all 25 proteus isolates at a rate of 100%, The results were consistent with what was reported in (Al-Mayahi, 2017) about the 100% isolation of *P. mirabilis* that contained mrpA genes.

The primary component of flaA, a virulence factor that aids this bacteria in spreading swiftly and causing illness, is a protein known as flagellin (Umpiérrez *et al.,* 2013). The development of flagella, which is controlled by several genes on the proteus chromosome, is a crucial aspect of motility and swarming. The flagellin protein FlaA, which is encoded by flaA, makes up the majority of the filamentous part of the *P. mirabilis* flagellum (O'May et al., 2008). Since 25 proteus species isolates were found to have the flaA gene at a ratio (100%) in the current study of the genetic aspect of flagella using a molecular technique, the findings

were somewhat similar to those of (Bunyan & Albakery, 2021) where the ratio (100%).

Conclusion

The virulence of proteus isolates was regulated by genes, and PCR amplification revealed that 25 proteus isolates had the mrp, flaA, ureC, and zapA genes.

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